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Field characterization of maize photosynthesis response to light and leaf area index under different nitrogen levels: a modeling approach

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Field characterization of maize photosynthesis response to light and leaf area index under different nitrogen levels: a modeling approach

by

Laila Alejandra Puntel

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

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Major: Crop Production and Physiology

Program of Study Committee:
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Ames, Iowa

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Laila A. Puntel

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GENERAL INTRODUCTION

Understanding yield variability within maize fields has become one of the most intriguing problems in current production research in the Midwestern US (Batchelor *et al.*, 2002). Evidence from nitrogen (N) fertilizer response trials suggests that there is a great deal of variability in the amount of N supplied to a corn crop by the soil. High yields with no N fertilizer applied are not uncommon (Bundy and Adraski, 1995), and the amount of additional yield that can be produced due to N fertilizer is highly variable from field to field (Lory and Scharf, 2003).

Nitrogen use is an issue of great concern in maize production, as the negative impact on groundwater quality and the relationship with an increase in nitrous oxide emissions (a potent greenhouse gases), has become a public issue (e.g., Cerrato and Blackmer, 1991; Klausner *et al.*, 1993; Schlegel *et al.*, 1996, Millar *et al.*, 2010). We require various and renovated tools for a more precise assessment of N requirements (Batchelor *et al.*, 2002).

Crop growth models can provide a tool for greater understanding of the responses of yield to different N levels observed experimentally (Sinclair and Amir, 1992). The strength of these models is their ability to account for stress by simulating the temporal interaction of stress on plant growth each day during the season (Batchelor *et al.*, 2002).

Knowledge of the factors governing N demand is essential to predict the needs of crops under a wide range of field situations (Greenwood, 1982; Van Keulen *et al.*, 1989). A functional approach to estimate the actual demand of the crop is to consider a detailed characterization of the effect of N on the photosynthetic machinery of the leaves (Grindlay, 1997) that can be included into leaf-based photosynthesis models. For improved predictions from these models, an accurate estimation of leaf area index is an important component to be estimated under different N levels (Lizaso *et al.*, 2005).

The first objective of the present work was to characterize the photosynthetic response to light under different N levels throughout the growing season. The second objective was to incorporate

in a crop growth model a dynamic relationship between photosynthetic parameters and leaf N concentration taking in consideration the effects of developmental stage following the results from the first objective.

A better prediction of the N-limited LAI and canopy photosynthesis by crop models would eventually lead to a more accurate assessment of N supply and demand in the cropping systems that might provide a powerful opportunity for mitigating nitrous oxide emissions from agricultural soils and leached N loss as well.

CHAPTER 1

FIELD CHARACTERIZATION OF LEAF AREA INDEX AND PHOTOSYNTHETIC LIGHT CURVES OF MAIZE GROWN UNDER DIFFERENT N RATES

1. Introduction

The yield (Y) of a crop, per unit of area, in a given period of time can be expressed as:

$$Y = Q * I * \epsilon * H \quad \text{Eqn. [1]}$$

where Q is the total quantity of incident solar radiation received over a period of time and area (MJ m^{-2}); I is a fraction of Q that is intercepted by the crop; ϵ is the efficiency with which that intercepted radiation is converted into total plant dry matter; and H is the harvest index or the efficiency with which biomass is partitioned into the harvested product (Monteith, 1977).

Q mainly depends on the latitude and the season and may vary with weather factors such as cloudiness. I is affected by the leaf area index (LAI) of the crop and canopy structure and architecture. The efficiency with which the intercepted radiation is converted into biomass (ϵ) is determined primarily by a combined photosynthetic rate of all the leaves within the canopy minus the losses by crop respiration (R).

Insufficient nitrogen (N) affects the final yield (Y) of a crop through a reduction in resource capture, resource use efficiency, or both (I and ϵ , respectively Eqn. 1). The first effect, a reduction in the amount of photosynthetic active radiation (PAR) intercepted by the canopy is a consequence of a reduction, under N stress, in the leaf expansion rate (Muchow, 1988; Gastal *et al.*, 1992; Gastal and Nelson, 1994) resulting in a decrease in LAI (Colnenne *et al.*, 2002). The second effect, a reduction in the radiation use efficiency (RUE) (Muchow and Davis 1988; Colnenne *et al.*, 2002) is due to a decrease in the leaf N content per unit leaf area (SLN), which can adversely affect the rate of

photosynthesis (Sinclair and Horie, 1989; Muchow and Sinclair, 1994; Vos and van der Putten, 1998).

One way to capture and describe the effect of N on canopy photosynthesis is by characterizing the photosynthetic light response curve (Fig. 1) at different N levels. Integration of photosynthesis from leaf to canopy levels should account for variation of photosynthetic responses to Photosynthetic Photon Flux Density (PPFD, Fig. 1) as well as to leaf N and other environmental variables (such as temperature and CO₂ gradients). In the majority of photosynthetic scaling models, the leaf photosynthetic ‘light response’ is the most empirical part of these models. Predictions of canopy photosynthesis are very sensitive to parameters describing the photosynthetic response of leaves to PPFD (Kull and Kruijt, 1998).

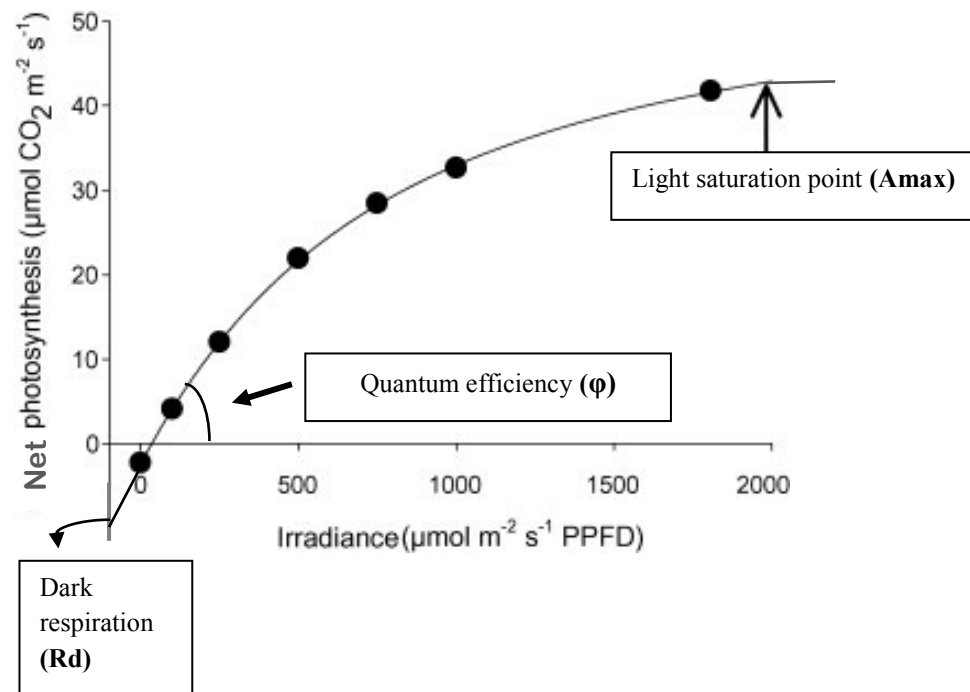


Fig. 1 Representative photosynthetic light response curve measured with an infra-red gas analyzer on maize. Adapted from Fletcher *et al.*, (2008).

In the simpler cases, photosynthetic light response is described by empirical equations like the rectangular or non-rectangular hyperbola (Hirose and Werger, 1987; Kull and Jarvis, 1995). In other cases, this response is described partly mechanistically with limitations from different components of the photosynthetic apparatus (Collatz *et al.*, 1992; Von Caemmerer and Farquhar, 1981).

In general, photosynthesis response to light has been described with models that include an asymptote of the curve representing the maximum assimilation rate in $\mu\text{mol m}^{-2} \text{s}^{-1}$ (A_{max}), an initial slope of the response to light as $\mu\text{mol CO}_2$ per μmol quanta (apparent quantum efficiency, ϕ), and dark respiration (R_d) expressed in $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 1).

Many simple canopy photosynthesis models (Hay and Porter, 2006) rely on the assumption that the photosynthetic light response does not change throughout the canopy and that all leaves on average operate at the same relative position along the response curves (e.g. Sellers *et al.* 1992; Kull and Jarvis, 1995). The variability of these parameters during the growing season has not been well described in maize. In addition to their change with time, it is expected these parameters vary depending on the leaf N concentration.

Many studies have shown that N deficiency significantly decreases the CO_2 assimilation capacity of the plants (Lu and Zhang, 2000). Nitrogen mainly reduces the light-saturated photosynthetic rate and has a small effect on ϕ (Lawlor, 1995). The current literature does not characterize well the effect of N on photosynthetic parameters throughout the entire growing season.

Low CO_2 assimilation capacity is associated with a decrease in Ribulose 1, 5-bisphosphate carboxylase (Rubisco) content (Ferrar and Osmond 1986; Evans, 1989) and its activity (Terashima and Evans, 1988), as well as a decrease in the synthesis of several key enzymes involved in the Calvin cycle (Seemann *et al.*, 1987). Particularly, in developing maize leaves N deficiency has been

found to result in a significant reduction of phosphoenolpyruvate carboxylase (PEPc), pyruvate orthophosphate dikinase (PPDK) and Rubisco (Sugiharto *et al.*, 1990).

On the other hand, the effect of N deficiency on ϕ has been linked to damage in photosystem 2 (PSII) (Nunes *et al.*, 1993; Verhoeven *et al.*, 1997) or with variation in bundle sheath leakiness to CO₂ (Meinzer and Zhu, 1998). Other studies have demonstrated, however, that N deficiency has no effect on ϕ and resulted in no damage to PSII (Khamis *et al.*, 1990; Sugiharto *et al.*, 1990; Wang *et al.*, 2012).

Quantum efficiency has shown large variability and causes of this variability are not very well understood (Skillman, 2008). Characterization of ϕ becomes more important when integrated over a day or longer growth periods, especially when leaf area index (LAI) is high and a large proportion of the leaves are shaded and operating at low irradiance (Gastal and Lemaire, 2002).

A lack of association between dark respiration (Rd) and leaf N concentration has been found for C₄ species such as *Amaranthus retroflexus*, *Panicum maximum* (Wilson, 1975; Byrd *et al.*, 1992) or maize (Wang *et al.*, 2012) in greenhouse studies. To our knowledge, studies on Rd in maize grown under field conditions are lacking and it has not been estimated at different phenological stages during the growing season.

Additionally, there is limited information about the effect of growth stages on the leaf photosynthesis response of maize through the growing season; measurements of this nature are rarely carried out under field conditions (except Moreno-Sotomayor *et al.*, 2002). Most measurements of photosynthetic parameters on maize have been done in laboratory or greenhouse conditions on leaves corresponding to early stages of the plant development (Jacob and Lawlor, 1991; Dai *et al.*, 1995). The first leaves to appear (leaves 1 through 6, for example) differ from the upper ones (most recently expanded) (Bos *et al.*, 2000) and usually are non-functional during most of the season (Wolfe *et al.*, 1988).

To understand when and why the shape of light response curves varies, we need to assess how these parameters (A_{max} , ϕ , and R_d) vary with leaf N concentration and developmental stage. The objective of this study was to characterize the photosynthetic response of field grown maize leaves at different growth stages (V4 to R5) in response to three different N treatments (N0, N90 and, N225). Additionally, information of LAI, leaf dimensions and light interception at different N treatments was assessed to better understand changes in resources capture (I, eqn. 1).

In general, photosynthetic parameters are assumed to remain constant throughout the growing season or inferred from the literature (Amthor *et al.*, 1994). Results from the present study may expose the dependence of photosynthetic parameters on leaf N concentrations at different developmental stages in maize growth models.

2. Material and methods

A field experiment was conducted at the Agronomy Research Farm, Iowa State University (site11, 42°1' N, 93°45' W) during 2011 on a Clanisteo loam soil (Fine-loamy, mixed, superactive, calcareous mesic, typic Eudoaquoll).

The commercial maize hybrid Pioneer 461 (P0461XR) was sown on 10 May 2011 with a plant density of 9 plants m⁻². The experiment was set in a randomized complete-block design with three replications. Within the blocks three N treatments were randomly assigned to plots comprised of eight 15 m long rows with a row spacing of 76 cm. Nitrogen fertilizer treatments were a no-N control, 90, and 225 kg N ha⁻¹ (further listed as N0, N90, N225, respectively) as side-dress coulter-injected urea-ammonium nitrate (UAN) applied shortly after planting. Soil samples were taken before planting to determine routine tests with phosphorus (P) and potassium (K) applied as needed to ensure maximum yields. Pest, diseases, and weeds were adequately controlled.

2.1 Measurements

2.1.1 Light interception and LAI

Incident photosynthetic active radiation (PAR, I_o), the amount of PAR transmitted through the canopy (I_{tr}) and, the fraction of PAR intercepted by the canopy (Θ) were measured several times during the growing season. Measurements were obtained by placing diagonally a line quantum sensor (AccuPAR LP-80; Decagon Devices) below the canopy in two randomly selected areas in each plot between 10 and 14 h on clear-sky days. A single measurement consisted of three observations across a 1-m transect. The amount of I_o was automatically recorded with each below-canopy measurement. The fraction of I_o intercepted by the canopy was calculated as $\Theta = (I_o - I_{tr}) / I_o$.

Leaf Area index (LAI) was estimated indirectly and non-destructively using a line ceptometer quantum sensor (AccuPAR LP-80; Decagon Devices).

In addition, the leaf length (LL) and maximum leaf width (at the widest point; LW) was measured to estimate the area of individual leaves from the three different N treatments. Measurements were performed on fully expanded leaves. Individual leaf area was calculated according to (Montgomery, 1911):

$$\text{Individual leaf area} = 0.75 * LL * LW \quad \text{Eqn. [2]}$$

Total number of leaves was 19 and the last leaf for the N0 treatment was not measured for length and width.

2.1.2 Corn phenology

Phenological growth stages were recorded weekly for 10 tagged plants in each plot, following Abendroth *et al.* (2011). This method determines leaf stage in corn by counting the number of leaves on a plant with visible leaf collars, beginning with the lowermost, short, rounded-tip true leaf and ending with the uppermost leaf with a visible leaf collar. The leaf collar is the light-colored collar-like “band” located at the base of an exposed leaf blade, near the spot where the leaf blade comes in contact with the stem of the plant. Leaves were marked as collared leaves for subsequent staging.

Silking dates were determined when 50% of the plants had visible silks and anthesis dates when 50% of the plants started to shed pollen.

The time of physiological maturity was determined by assessing the presence of black layer at the base of the grain. This black layer indicates that no further accumulation of grain mass is possible (Daynard and Duncan, 1969).

2.1.3 CO₂ gas exchange measurements

A portable infra-red gas analyzer (LI-COR 6400, Lincoln, NE) with a closed configuration was used to measure photosynthesis response curves to light. Measurements were performed for all N treatments at several growth stages. From V4 to V14 stages light curves were measured at the last fully expanded leaf and from R1 to R5 measurements were recorded always on the ear leaf (leaf 14). The leaf area clipped by the chamber was 6 m^2 at halfway along the length of the leaf. Light curves were measured between 10 h and 14 h during clear-sky days (Dohleman and Long, 2009).

The light source provided by the equipment enable for automatic changes of the PPFD, with 3 to 5 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ intervals, which was used for measurements of photosynthetic light responses. The PPFD chosen ranged from 50 to $2300 \mu\text{mol m}^{-2} \text{ s}^{-1}$ in nine steps; the initial level of light in the chamber was set similar to the ambient light conditions. Temperature of the gas exchange cuvette block was set to the outside air T recorded at the start of the measurement cycle using the leaf thermocouple junction of the gas analyzer. Relative humidity (RH) was held constant for the duration of each measurement cycle, regardless of short-term fluctuations in air humidity. Reference CO_2 in the cuvette was set to match the external air concentration (approx. $400 \mu\text{mol mol}^{-1}$) and the flow was set to 300 mmol s^{-1} . Measurements were recorded once CO_2 uptake and stomatal conductance stabilized within the chamber (Dohleman and Long, 2009).

Additional photosynthesis light response curves were conducted when nearly 100% of the plants were at R2 and R3. These stages were labeled R2b and R3b, respectively.

2.1.4 Nitrogen samples

Total leaf N concentration (dry matter basis) was determined using a rectangular sample from the same leaf section where the photosynthesis-light curves were made. The area of the rectangular section was enough to collect 1 g of dry matter. During V4 stages, more than one middle section of the leaf was sampled to conduct the N analysis. Samples were dried at 60°C for 72 h, weighed, and

finely ground to determine total N concentrations by dry combustion at 950°C with a LECO TruSpec CN, LECO, St. Joseph, MI (LECO, 2008).

2.2 Analysis of Data

2.2.1 Photosynthetic model and parameter optimization

Light curves were obtained by fitting the data to a C_4 photosynthesis model (Collatz *et al.*, 1992). The model uses simple biochemical intercellular transport including inorganic carbon fixation by PEP carboxylase, light dependent generation of PEP and Rubisco, Rubisco reaction kinetics, and the diffusion of inorganic carbon and oxygen between the bundle sheath and mesophyll. Tightly coupled with this C_4 photosynthesis model is a version of the Ball *et al.*, (1987) model of stomatal conductance Eqns. (A1)-(A3).

The fitted model was used to determine three main parameters; A_{max} (the maximum assimilation rate under saturated light intensities), ϕ (apparent quantum efficiency) and, R_d (mitochondrial dark respiration). Photosynthesis rate is predicted as a function of T , light, internal CO_2 (C_i , $\mu\text{mol mol}^{-1}$) and RH. Equations in the model represent an analytical solution to the coupled C_4 photosynthesis-stomatal conductance model in which these three potentially rate limiting conditions are expressed as a quadratic which may be solved to give leaf-level predicted assimilation rate in terms of variables C_i , O_i and I_{abs} (Collatz *et al.* 1992) Eqns (A1)-(A3).

Parameters of the photosynthesis model were optimized by minimizing the sum of square deviations from observed and simulated. The algorithm used to find the parameters that minimize the least-squares criteria was 'Nelder-Mead' as implemented in the `optim` function in R (R Development Core Team, 2011).

2.2.2 Statistical analysis

Photosynthetic parameters obtained from V4 to V10 were grouped as vegetative observations (V) and observations obtained from the leaf 14 (V14 to R5 stage) were grouped as V14 for the statistical analysis. Data analysis was performed by linear regression and analysis of variance procedures of SAS MIXED (SAS Institute, Cary, NC). When main or interaction effects were significant, a linear contrast was used for comparisons among means. Data for 2 sub-sampled plants were averaged together for each N treatment and sample period throughout the growing season.

3. Results

The N treatments applied in this study significantly affected grain yield (Table 1). N0 treatment had the least yield compared to N90 and N225. Grain yields for N90 and N225 were 5095 and 6638 kg ha⁻¹ greater than N0. There were significant differences in grain yield between N90 and N225 ($P<0.05$).

Nitrogen treatments greatly affected leaf and stem biomass allocation (Table 1). Application of 90 or 225 kg of N per ha produced more dry matter production of leaves and stems than the control (N0). There were no differences, however, between N90 and N225 for leaves and stem biomass production ($P<0.05$).

Table 1 Mean dry biomass (Mg ha⁻¹) and standard error (n=18) for leaves, stem and grain at R6 stage for three different N treatments (N0, N90, and N220). Different letters indicate significant differences between means ($P<0.05$)

Treatment	Leaves	Stem	Grain
	-----Mg ha ⁻¹ -----		
N0	2.9a	5.3a	7.2a
N90	3.4b	6.1b	12.3b
N225	3.6b	6.7b	13.8c

3.1 Leaf area index (LAI), individual leaf area and, light interception

Leaf area index gradually increased during the growing season and mean values for N90 and N225 were significantly greater in comparison with the control (Fig. 2). During reproductive stages, significant differences were found between N90 and N225 ($P<0.05$). At the end of the growing season, the LAI was maintained around 4.5 and 5.5 for N90 and N225, respectively, while under deficient N conditions LAI dropped to slightly less than 3 (Fig. 2).

The area of a fully developed leaf was dependent on the leaf position and N treatments (Fig. 3). Generally, leaf area increased from the basal leaf positions up to leaf 12 and decreased again from leaf 13 towards the apical leaf positions. Leaf area for N90 and N225 was significantly greater than N0 for leaf 10 to 18. In addition, N225 had more leaf area than N90 for leaves 12 and 18.

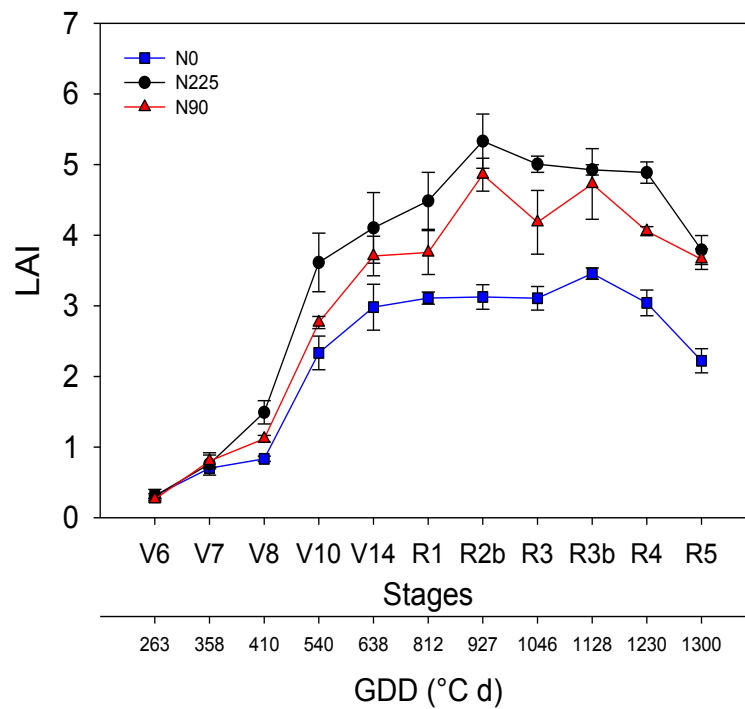


Fig. 2 Relationship between leaf area index (LAI in $\text{m}^2 \text{m}^{-2}$) and growing degree days (GDD in $^{\circ}\text{C d}$ from emergence, $T_{\text{base}} = 10^{\circ}\text{C}$) for maize grown at three different N treatments (N0, N90, and N225 rates). Vertical bars represent the standard error of the mean ($n = 6$).

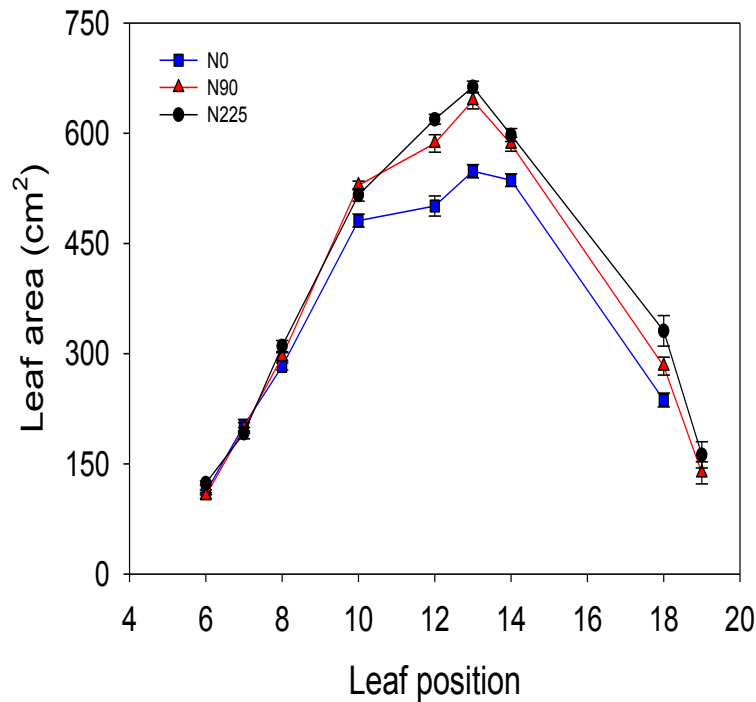


Fig. 3 Area of individual leaves at different positions in maize plants grown at three different N treatments (N0, N90, and N225 rates). Vertical bars represent the standard error of the mean (n =18).

The proportion of incident PAR intercepted by the canopy increased as LAI increased during the season until R1 when the canopy reached the maximum light interception (maximum LAI). Light interception was significantly affected by the N treatment (Fig. 4). The N0 treatment intercepted similar proportions of light as N90 and N225 until V8 (410 °C d). After V8, differences in light interception increased, especially at the end of the growing season. The N90 and N225 treatments intercepted a greater proportion of light than did N0 from V8 to R5 (410 to 1300 °C d). Significant differences between N90 and N225 were found for V8, V10, and R4. Almost 95% of the incident PAR was intercepted under N225 treatment around flowering (R1). Light interception around flowering was 86 and 90 % for N0 and N90, respectively.

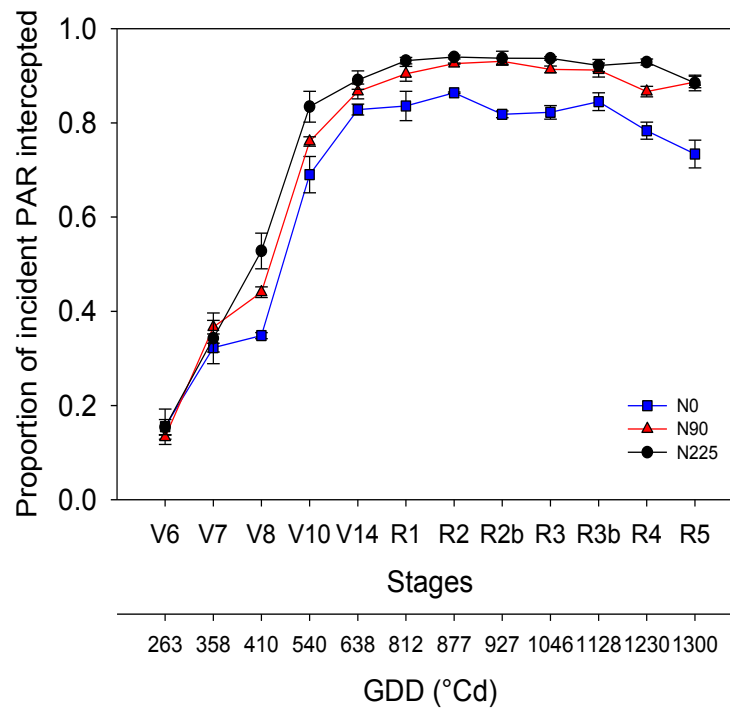


Fig. 4 Proportion of incident PAR intercepted by maize canopy at three different N treatments (N0, N90 and, N225 rates). Vertical bars represent the standard error of the mean (n =18).

Leaf N concentration declined after V7 (360 °C d) until R5 (1300 °C d) (Fig. 5). Values at V4 (230 °C d) were statistically lower for N0 than N225. In addition, this decline in leaf N concentration after about 400 °C d followed the same general pattern for all the N treatments. There were significant differences between N90 and N225 compared to the control after 400 °C d ($P < 0.001$) except for late in the season (1300 °C d) where N90 did not differ from N0. Significant differences were found between N90 and N225 at the end of the growing season (°C d > 1100) where at higher rates of N fertilization the leaf N concentration was maintained at a value close to 30 g kg⁻¹ but at N90 the leaf concentration decreased to slightly above 20 g kg⁻¹ ($P < 0.001$).

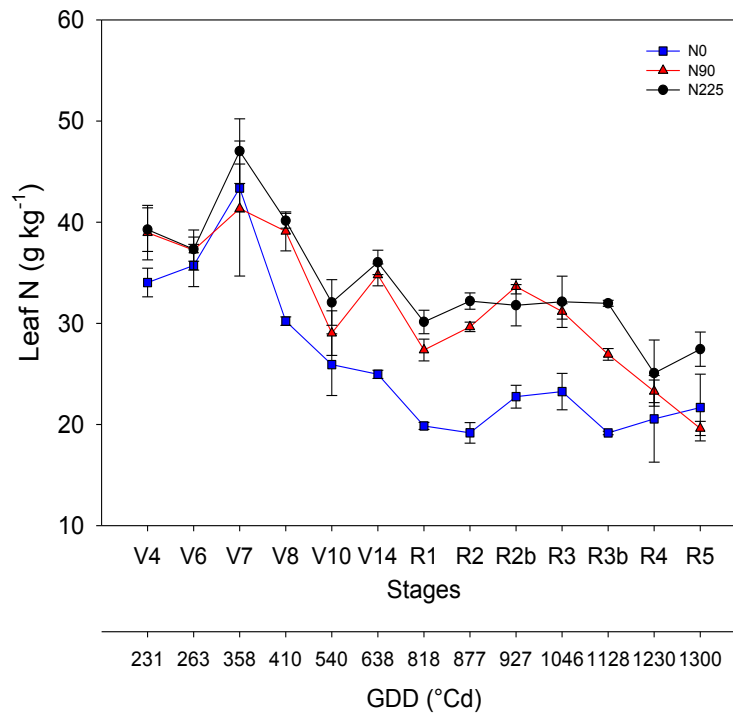


Fig. 5 Relationship between leaf N concentration (kg N kg^{-1}) and growing degree days (GDD in $^{\circ}\text{C d}$ from emergence, $T_{\text{base}} = 10^{\circ}\text{C}$) for maize grown under three different N treatments (N0, N90, and N225 rates). Vertical bars indicate SE of the means ($n=6$).

3.1 Effect of leaf N concentration on photosynthetic parameters

Corn plants grown under different N application rates had leaf N concentrations ranging from 15.1 to 46.6 (g N kg^{-1}) and A_{max} from 18.2 to 59.6 $\mu\text{mol m}^{-2} \text{s}^{-1}$. During V stages A_{max} and leaf N concentration reached the highest values of the season (59.6 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 46.6 g N kg^{-1} , respectively). The photosynthetic saturation rate was higher for V than V14 stages even at leaf N concentrations similar to V14 measurements.

The dependence of A_{max} on leaf N concentration was found to be linear during V and V14 (Fig. 6A). The response of A_{max} to leaf N concentration was 0.611 and 0.865 $\mu\text{mol m}^{-2} \text{s}^{-1}$ per 1 g N kg^{-1} increased for V and V14 stages, respectively. The interaction between leaf N concentration and development phase (V and V14) was not significant.

During V14 stages, Amax values for N225 differed from the control in $7 \pm 2.09 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively (Fig. 7A). In addition, differences in Amax between N225 and N90 were significant for R1, R3b, and R4 stages ($P < 0.05$). During V stages, in contrast, there were no significant differences between N treatments.

In this study, variation in apparent quantum yield (ϕ) was not affected by leaf N concentration (Fig. 6B). Apparent quantum efficiency varied from 0.047 to 0.082 $\mu\text{mol mol}^{-1}$ with leaf N concentrations. On average, ϕ for vegetative and reproductive phases was $0.0635 \pm 0.0081 \mu\text{mol mol}^{-1}$.

There was a significant effect of leaf N concentration on Rd. Observations demonstrated, however, a weak association (Fig. 6C). Dark respiration tended to be low at low leaf N concentration for the V14 leaf and high during V stage with high leaf N concentration, but differences between V14 and V stages were not significant (Fig. 7C).

The relationship between Amax-Rd ratio and leaf N concentration for V and R stages was found to be not significant (Fig. 6D). There were no significant differences between vegetative and reproductive stages. The mean value for all the observations was 17.21 ± 0.66 .

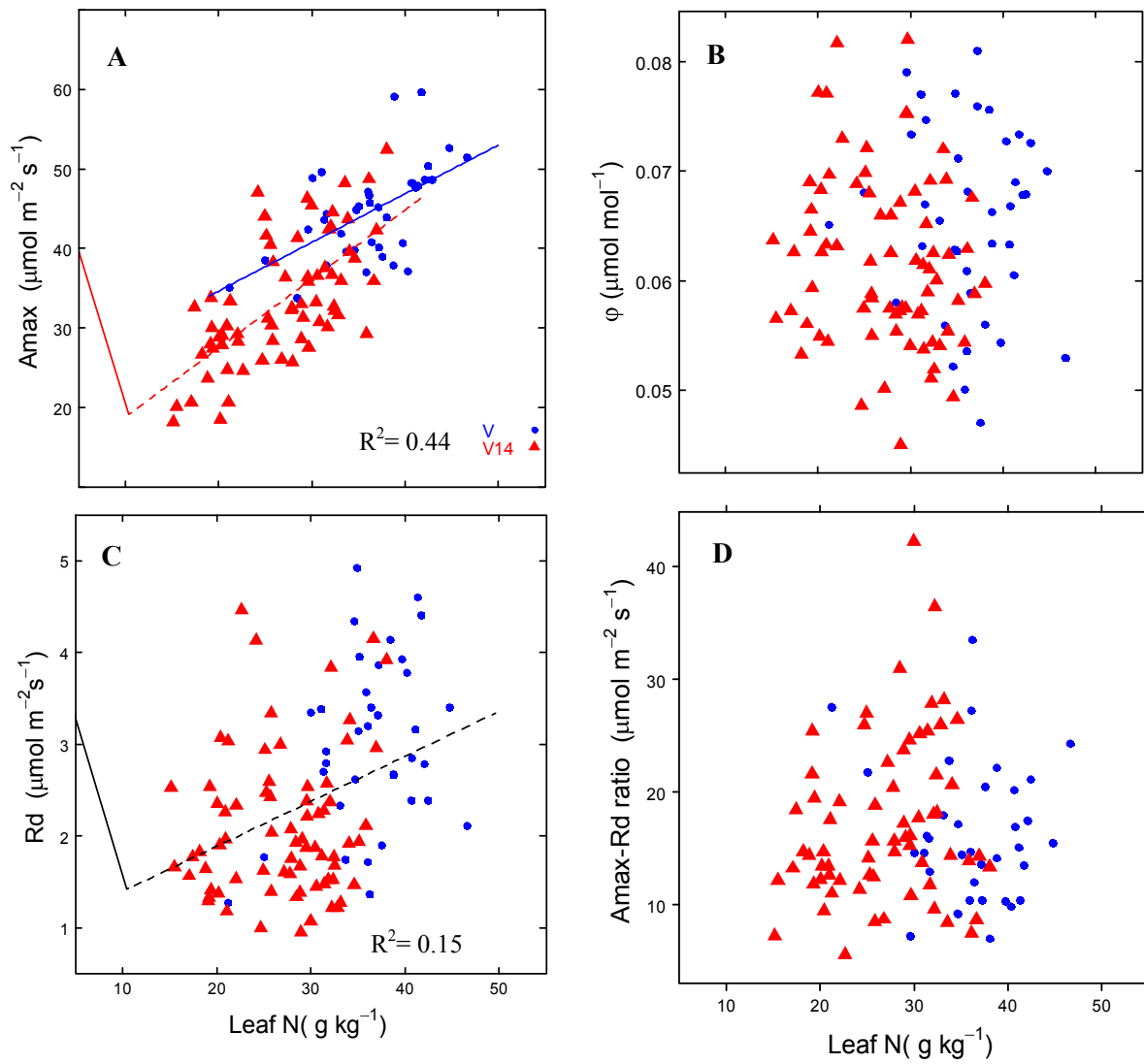


Fig. 6 Photosynthetic parameters and Amax-Rd ratio in relation to leaf N concentration for vegetative (V, triangles) and V14 leaf (dots) for corn grown at three levels of N (N0, N90, and N225). A: Amax; V (continuous line): $y=22.44 + 0.611x$; V14: $y=10.21 + 0.865x$. B: Apparent quantum efficiency for CO₂ uptake (ϕ). C: Dark respiration (Rd); $y=0.91 + 0.049x$. D: Ratio between Amax and Rd.

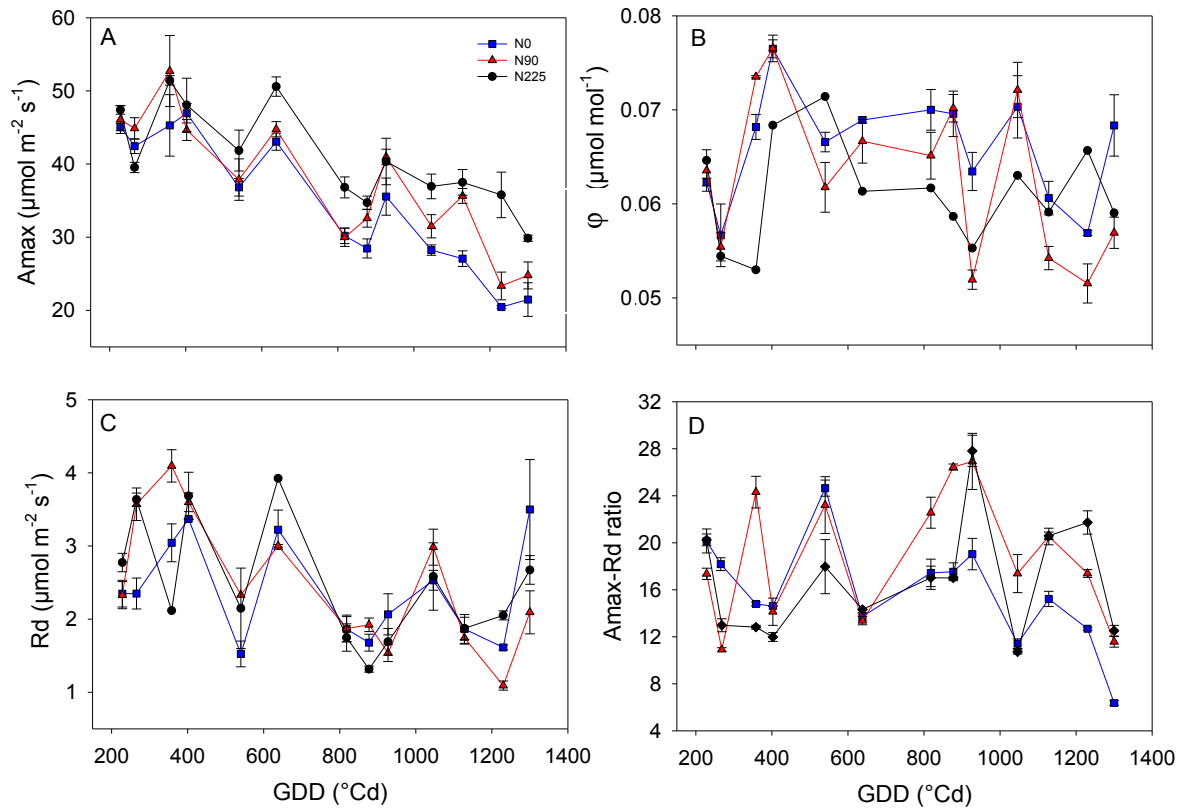


Fig. 7 Relationship between photosynthetic parameters and Amax-Rd ratio and growing degree days (GDD in °C d from emergence, Tbase= 10 °C) for maize grown under three different N treatments (N0, N90, and N225 rates). A: Amax B: Apparent quantum efficiency for CO₂ uptake (φ) C: Dark respiration (Rd) D: Ratio between Amax and Rd. Vertical bars indicates SE of the means (n=6).

The ratio between Amax and Rd decreased during reproductive stages (°C d > 800) (Fig. 7D). Amax-Rd ratio decreased to 6.34 at the end of the growing season (1300 °C d) at N0 treatment, while Amax-Rd ratio for N90 and N225 treatments was significantly higher than the control ($P < 0.001$). During vegetative stages (°C d < 800) Amax-Rd ratio did not show any particular pattern.

4. Discussion

Low N supply reduced biomass accumulation in leaves, stem and grain (Table 1). The reduction in biomass accumulation induced by N deficiency (e.g. N0) was associated with a decrease in individual leaf area (Fig. 3), leaf area duration (Fig. 4) and photosynthesis per unit of area (Fig. 6 and 7) (Paponov and Engels, 2003).

A reduction in LAI produced by N stress (e.g. Fig. 2) decreased the ability of the crop to intercept a sufficient amount of radiation during the critical period (15d before and after flowering) where 95% of the radiation should be intercepted to maximize growth rate per plant. The definition of the potential kernel number, the main component of maize yield, is strongly related to the light intercepted during the critical period and thus growth rate per plant (Andrade *et al.*, 1993; Otegui *et al.*, 1995). Light intercepted by N0 and N90 during the critical period were lower than 95% (Fig. 4); in contrast N225 canopy intercepted almost 95% of the incident PAR. Low growth rates produced by the decreased in light interception in N0 and N90 treatments during the critical period may explain why the final yield of N90 and N0 were 5.1 and 6.6 Mg ha⁻¹ lower than N225, respectively. The LAI, leaf area of individual leaves, and the proportion of incident PAR intercepted, however, were not significantly different for N90 and N225. This suggests that maize plants growing under N90, maintained the LAI and hence light interception (I, Eqn. 1) but they certainly reduced the concentration of leaf N (Vos *et al.*, 2005, Fig. 5). Consequently, the canopy was intercepting similar amounts of PAR but the photosynthetic capacity was lower (Fig. 6A). Vos *et al.*, (2005) found that maize strives for maintenance of leaf area per leaf at the expense of decreased N concentrations per unit of leaf area and decreased photosynthetic capacity.

Our results indicated that LAI was mainly affected by a reduction in the final area of individual leaves (Uhart and Andrade, 1995; Trapani and Hall, 1996). Changes in N rates mainly affected leaf expansion, whereas the rate of leaf appearance and leaf numbers was not affected (Muchow, 1988; Paponov and Engels, 2003). Snir and Newmann (1997) showed that the decrease in leaf elongation induced by low N was caused by a reduction in cell elongation and final size of epidermal cell, while cell production was not affected.

Leaf area index was found to reach maximum values after R1 (4.0, 5.3, and 6.0 for N0, N90, and N225 treatments, respectively). Increases of LAI after R1 stage, however, are not likely. According to LAI measurements, differences between N90 and N225 treatments were significantly different during reproductive stages. According to individual leaf area measurements, however, differences between N90 and N225 were not significant during the entire season. This disagreement may be caused by a methodological issue in LAI measurements during reproductive stages. The indirect method used for measuring LAI by comparing differential light measurements above and below canopy might not be accurate. The maximum measurable LAI is generally lower for devices measuring gap fraction than the one assessed via direct methods, with LAI reaching an asymptotic saturation level at a value of about 5. The likely cause is gap fraction saturation as LAI approaches 5–6 (Gower *et al.*, 1999).

Leaf nitrogen concentration decreased during the growth cycle for all N treatments (Fig. 5), which is consistent with other studies (e.g. Plénet and Lemaire, 2000). External supply of N to the plants is not the only source that alters leaf N concentration at the leaf level during the growing season. Changes in N accumulation are highly related to the crop growth rate and to biomass accumulation. For example, shaded leaves at the bottom of the canopy require less N to maximize carbon assimilation due to light attenuation within the canopy (Gastal and Lemaire, 2002). The effect of the leaf age on N distribution and Amax appears to be more limiting than the light acclimation

effect, though it is recognized that both effects occur concurrently (Hikosaka *et al.*, 1994; Schieving *et al.*, 1992).

Under low N availability, the leaf N concentration decreased more than at high N supply after 400 °C d. This can be explained by the strong demand experienced by the crop around the pre-anthesis phase when plants are actively developing leaf area and root systems (Lemaire and Gastal, 2009). Leaf N concentration values were similar for N90 and N225 until the end of the growing cycle where leaf N concentration in N90 tended to decrease more than N225. The ability of the N225 treatment to maintain a higher leaf N concentration may be due to the buffer N storage in the stems that is remobilized at the end of the growing season, keeping the leaf active and green longer (Gallais and Hirel, 2004).

It is well known that leaf N content declines during later stages of plant development and the effects of this decline on the photosynthetic parameters must be accounted for (Lindquist and Mortensen, 1999). In this study, Amax was lower at N0 than N90 and N225 (Fig. 7A) in line with literature (Muchow and Sinclair, 1994; Vos and van der Putten, 1998). Even though leaf area formation is more sensitive to N deficiency than the rate of net photosynthesis (Radin and Boyer, 1982), reductions in photosynthesis rate can strongly affect canopy photosynthesis. The decrease of CO₂ assimilation capacity is likely associated with a decrease in Rubisco content (Ferrar and Osmond, 1986), which in C₄ plants constitutes 30% of the soluble proteins (Sugiyama *et al.*, 1984) and 5 to 9% of the total leaf N (Sage and Sharkey, 1987; Makino *et al.*, 2003).

Further, reduction in photosynthetic capacity under limiting N conditions has been related with a significant reduction of phosphoenolpyruvate carboxylase (PEPc), pyruvate orthophosphate dikinase (PPDK) and Rubisco (Terashima and Evans, 1988; Sugiharto *et al.*, 1990).

The relationship between Amax and leaf N concentration was found to be linear in V14 leaf and V stages (Fig. 6A) in accordance with numerous studies (Wolfe *et al.*, 1988; Sinclair and Horie, 1989; Mcculloughl *et al.*, 1994; Vos *et al.*, 2005; Paponov *et al.*, 2005). Light-saturated

photosynthetic rates measured on V14 leaf were not as high as those obtained during V stages at same levels of leaf N (Fig. 6A). Similar results for maize were observed by Moreno-Sotomayor *et al.* (2002) where Amax at V13 stage was higher than the rest of the reproductive stages (R1 to R5). Particularly during reproductive stages, sunlight distribution is being attenuated from the top to the bottom of the canopy as a result of the increase in the leaf area developed over the season (e. g. LAI \cong 4, \cong 800 °C d; Fig. 2). Leaves exhibit a structural and functional acclimation of the photosynthetic apparatus to the light intensity changes experienced during their growth (Reyss and Prioul, 1975; Prioul *et al.*, 1980; Bjorkman, 1981). The acclimation of the photosynthetic apparatus under low light intensities has a detrimental effect on the light-saturated rate of CO₂ uptake (Boardman, 1977). In addition, recent research has reported that the decline in Amax over the growing season was not related with leaf thickness or other anatomical features, but probably because of biochemical aspects of photosynthesis (Moreno-Sotomayor *et al.*, 2002). This information suggests that ear leaves measured during reproductive stages were experiencing lower light levels than the uppermost leaf at vegetative stages resulting in low values of Amax even at similar values of leaf N concentration.

The effect of leaf age on Amax was greater at lower N supply than at higher N supply, whereas the effect of N supply was smaller for leaves in V stages (Yin *et al.*, 2011). At reproductive stages and high nitrogen levels (N225) leaf N concentration was not lower than 25 g N kg⁻¹ while under N0 treatment leaf N concentration was reduced to 15.5 g kg⁻¹ resulting in Amax values of 28.41 and 18.21 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively (Fig. 6A and Fig. 7A). Greater post-silking N may result in a longer duration of leaf greenness and, consequently, higher CO₂ uptake (Echarte *et al.*, 2008).

Under N90 and N225 treatment the photosynthesis rate at saturation light was maintained at an average of 5 and 9 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively, higher than the control at the end of the growing season (1300 °C d) (Fig. 7A). Amax declined during reproductive stages, however, for both low and high N conditions and this is in agreement with previously reported data (e.g. Papanov and Engels,

2003; Ding *et al.*, 2005). Furthermore, it has been suggested that this ontogenetic decline of A_{max} is not only a consequence of reduced leaf N concentration but it is also related to quantity and activation state of carboxylating enzymes and sensitivity of stomata function (Hasegawa and Horie, 1996).

In this study, the observed low leaf N concentrations for N0 treatments during reproductive stages could be a result of the strong sink strength of the grain, creating a high demand of photosynthates and N compounds. Under N deficiency conditions, remobilization of N from the leaves to the grain greatly affects photosynthesis during grain filling (Weiland and Ta, 1990). In addition to limiting N conditions, the sink strength of the plant is decreased by a reduction in grain set (Uhart and Andrade, 1995). This might have produced carbohydrate accumulation in the leaves, leading to a feedback inhibition of photosynthesis (Krapp *et al.*, 1993).

Apparent quantum efficiency was not affected by changes in leaf N concentration due to different N rates applications or age (Fig. 6B and Fig. 7B). Thus, limited N nutrition in maize led to a significant reduction in the photosynthetic capacity (e.g. decreasing A_{max}) without a reduction in the quantum efficiency of the leaves when they are growing at low irradiance (Khamis *et al.*, 1990, Sugiharto *et al.* 1990).

Several studies in C_3 plants have addressed the response of ϕ to leaf N concentration (eg. (Müller *et al.*, 2005) temperature and light acclimation (e.g Schultz, 2003). In contrast, ϕ for C_4 species has received less attention, or contradictory findings have been reported about the response to leaf N concentration. Some studies have shown that N deficiency decreases the quantum yield of photosystem 2 (PSII) electron transport and the maximal efficiency of the PSII photochemistry, suggesting that N deficiency induces some damage to PSII (Nunes *et al.*, 1993; Verhoeven *et al.*, 1997), whereas other studies have demonstrated that N deficiency has no effect on the quantum yield of PSII electron transport and results in no damage to PSII (Khamis *et al.* 1990; Bungard *et al.*, 1997; Lu and Zhang, 2000; Lawlor, 2001).

Meinzer and Zue (1998) described a positive linear relationship between ϕ and leaf N concentration in sugarcane clones (C_4 species) grown at three levels of N availability and attributed this to variation in bundle sheath leakiness to CO_2 . Quantum efficiency, however, is highly species dependent possibly in relation to different partitioning of N between Rubisco and harvesting complex proteins (Evans, 1989). Furthermore, most published studies describing a link between ϕ and N deficiency were not performed under natural field conditions (e.g. greenhouse studies or in pots) (Nikiforou and Manetas, 2011). Recently, a greenhouse study showed a non-significant increase in quantum efficiency by the increase of N levels in the leaves (Wang *et al.*, 2012). Crops grown in greenhouses develop thin leaves (high specific leaf area, SLA) while field crops usually develop thicker leaves (low SLA values). This means that light absorbance in field grown leaves is greater, and any additional application of N might have only a very minor contribution to increase light harvesting (by changing leaf morphology) and thus changing quantum efficiency. On the other hand, leaves grown under controlled conditions have a much stronger response to N application by increasing light harvesting and consequently quantum efficiency. Such anatomical differences may explain the observed discrepancy between field and greenhouse studies on quantum yield and its response to N.

Apparent quantum efficiency did not show any particular pattern throughout the growing season and there were no significant differences between V stages and leaf 14. Mean values were $0.065 \pm 0.0014 \mu\text{mol mol}^{-1}$ and $0.062 \pm 0.0010 \mu\text{mol mol}^{-1}$ for V stages and leaf 14, respectively (Fig. 7B). Similar results were suggested by Moreno-Sotomayor *et al.*, (2002) where ϕ showed no significant relationship with leaf age, leaf position or any anatomical feature. Mean values reported by this author for leaves 13 and 17 were $0.0626 \pm 0.0166 \mu\text{mol mol}^{-1}$ and $0.0607 \pm 0.007 \mu\text{mol mol}^{-1}$, respectively. This non-significant difference between young and old leaves, however, suggests that ϕ decreases in old leaves (McCree 1972; Ku and Edwards, 1978; Moreno-Sotomayor *et al.*, 2002).

The mean value of ϕ reported for plants with C_4 photosynthesis pathways measured under ambient concentration of atmospheric CO_2 and O_2 was $0.057 \pm 0.006 \mu\text{mol mol}^{-1}$ (Skillman, 2008). Under non-stressed situations the ϕ of CO_2 uptake for C_4 plants is assume to be relatively stable ($0.06 \mu\text{mol mol}^{-1}$) over a range of 25 to 30°C (Skillman, 2008). In the present work, the overall average for ϕ ranged from 0.045 to $0.08 \mu\text{mol } CO_2 \text{ mol}^{-1}$ which is in agreement with published data (Fig. 6B). Variation for ϕ , however, can have a significant effect in overall canopy photosynthesis.

In maize, part of the variability in ϕ during the growing season has been explained by the effect of low temperatures, especially during early stages of the plant growth (Naidu *et al.*, 2003). Although temperature may account for some of the seasonal variation in quantum yield particularly on mornings of high radiation, the observed large variability in Fig. 6 suggested that other factors are influencing the values of ϕ (Fig. 6B). The minimum temperature of the previous day, analyzed as a possible variable affecting quantum efficiency, was not a significant effect. We hypothesize that other factors such as light acclimation, the age of the leaf, and leaf N are affecting the structural attributes of leaf morphology, and consequently the light absorption by the photosynthetic pigments (range 80 to 95%) affecting apparent quantum efficiency. Further research is still needed to address this issue.

Quantification of ϕ is affected by a number of factors (e.g. methodological issues, growing conditions). The observed variability in the ϕ (fig. 6B; 0.047 to $0.08 \mu\text{mol mol}^{-1}$) was not clearly explained by leaf N concentration, developmental stage or the minimum temperature of the day before measurements. Even under controlled conditions variability of ϕ is large and in general not well understood (Skillman, 2008). Until a better understanding of factors affecting the variability of ϕ is achieved, it seems reasonable to use a constant ϕ value for modeling maize photosynthesis.

It has been stated that canopy photosynthesis is rarely light saturated and the majority of the leaves operate at light levels considerably below those required to saturate CO_2 assimilation because of the combined effects of leaf angle and mutual shading (Baker *et al.*, 1988; Stirling *et al.*, 1991; Gastal and Lemaire, 2002). Although, this is clearly the case in a closed canopy (Inoue *et al.*, 1968) or

on cloudy days (Baker *et al.*, 1988), it may not be the case on cloudless days when the leaf area index (LAI) is less than 2. Photosynthesis at low light levels becomes more important when integrated over a day or longer growth periods, especially under a crop of high leaf area index where a greater proportion of the leaves are shaded (Gastal and Leamire, 2002).

There was a significant effect of leaf N concentration on Rd (Fig. 6C). This is in agreement with Osaki *et al.*, (2001) that found an increase in Rd as increasing leaf N concentration in maize. Byrd *et al.* (1992), however, found lack of relationship between Rd and leaf N concentration in a C₄ species (*Amaranthus retroflexus*) for mature leaves exhibiting a similar rate of decline after 16 h of darkness. In addition, a greenhouse study conducted by Wang *et al.* (2012) showed no significant effect of N treatments on Rd in maize. Therefore, the coupling of N and Rd of leaves of C₄ plants may be of only minor importance (Byrd *et al.*, 1992).

Dark respiration rate was higher, though not significant, during vegetative than V14 leaf (Fig. 7C) (Osaki *et al.*, 2001). High Rd values during vegetative stages could be explained by high amount of carbohydrates being synthesized under high light conditions (Byrd *et al.*, 1992).

Mean values for V stages and V14 leaf were 3.1 ± 0.185 and 2.25 ± 0.128 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. These values are consistent with published data by Dohleman and Long (2009) (3.21 to 3.57 $\mu\text{mol m}^{-2} \text{s}^{-1}$), although our data suggested that Rd for V14 leaf had lower values.

On the other hand, Rd values reported for maize grown in greenhouse conditions were lower than the ones obtained during vegetative stages in this study (2.325 ± 0.398 $\mu\text{mol m}^{-2} \text{s}^{-1}$, Yin *et al.*, 2011). Differences between the values obtained in this greenhouse study and the ones presented in this work could be explained by differences in light conditions during the leaves growth environment, frequently light conditions in greenhouse studies can be around 600 or lower $\mu\text{mol m}^{-2} \text{s}^{-1}$. It has been reported that low light conditions resulted in low values of Rd (Boardman, 1977).

Measurements were performed in already expanded leaves where expansion and growth are no longer occurring. Thus, the R_d estimated in this work might be mainly a measurement of maintenance respiration. It has been demonstrated that under high N rates R_d was higher but the increase was mainly due to increases in growth respiration and the percentage of maintenance was constant under high N rates (Byrd *et al.*, 1992). These authors attributed the increase of growth respiration at high N conditions to increases in meristematic activity rather than increases in leaf mass.

The relationship between A_{max} and R_d was not affected by leaf N concentration or developmental stage (Fig. 6D and 7D). A ratio of 17 ± 0.66 was obtained across N treatments and growth stages. Similar results were obtained by Byrd *et al.*, (1992) where the ratio between A_{max} and R_d was not affected by leaf N in maize (Fig. 6D). The A_{max} - R_d ratio reported by these authors was 12.2 with A_{max} of $35.3 \pm 3 \mu\text{mol m}^{-2} \text{s}^{-1}$ and R_d equal to $2.9 \pm 0.5 \mu\text{mol m}^{-2} \text{s}^{-1}$. The values found in the present work were higher than values obtained by Bird *et al.*, (1992), mainly due to high values of A_{max} .

Direct measurements of R_d are difficult to perform and they require sophisticated technologies (Pärnik and Keerberg, 2007). Several methods estimate R_d indirectly (Yin *et al.*, 2011) or is commonly fixed at 1% of A_{max} (Braune *et al.*, 2009). Dark respiration estimated in this study corresponded to almost 6% of V_{max} which is higher than the 1% proposed in the literature.

5. Conclusions

Changes in the ability of the crop to capture solar radiation (I, Eqn.) and to convert it into dry matter have been characterized for three different N treatments. Interception of solar radiation was affected by a decrease in LAI, mainly due to a decrease in individual leaf area, when no N or 90 kg ha⁻¹ were applied.

Results indicated that the light-saturated photosynthetic rate was linearly related with leaf N concentration. Furthermore, the response of A_{max} to changes in leaf N concentration was higher for the V14 leaf than V stages (V4-V10). The relationship between R_d and leaf N concentration was found to be significant. Apparent quantum efficiency (ϕ) did not show a relationship with leaf N concentration or developmental stage. The variability observed in this field experiments, however, suggested that more research is still needed to characterize changes in ϕ to estimate photosynthesis under low irradiance.

Detailed characterization of leaf area and photosynthetic response to light under different N levels are important factors in the estimation of canopy photosynthesis in crop growth simulation models that compute dry matter accumulation from temporal integration of canopy photosynthesis.

CHAPTER 2

CALIBRATION OF A PHOTOSYNTHESIS BASED MODEL (MAIZEGRO) WITH EMPHASIS ON LEAF AREA INDEX AND PHOTOSYNTHETIC PARAMETERS DEPENDENCE ON N

1. Introduction

Quantitative relationships between yield of maize and the application of N fertilizer to soils have been established in a large numbers of trials (e.g. Sawyer *et al.*, 2006). The relationships developed are invariably correlative, and extrapolation of these relationships to other environments, soils and management practices have been uncertain. Crop simulation techniques are increasingly used to support field research focused toward efficient and sustainable N use in cropping systems (Zhang *et al.*, 2002). This involves developing (or adapting) and assessing crop growth and N balance models for analyzing the effects of variation in climatic and N supply regimes on crop yields. Crop growth models can provide a tool for greater understanding of the responses of yield to different N levels observed experimentally (Sinclair and Amir, 1992).

In dynamic N approaches, two main aspects can be modeled separately: availability of mineral N to growing crops, and effects of crop N status on crop growth (Van Ittersum *et al.*, 2002). The establishment of a canopy that can efficiently absorb radiation and the conversion efficiency of the energy into biomass are key process in determining dry matter production. Understanding the role of N nutrition in canopy growth is a first step in modeling final crop yield in relation to N fertilizer inputs (Boote *et al.*, 1996).

There are crop growth models that follow a simple approach based on light intercepted and efficiency of conversion (I and E, respectively) to quantify daily rates of growth (Hay and Porter, 2006). Because radiation use efficiency (RUE) is not constant, such models generate inaccurate yield predictions under stress conditions. Models including dynamic simulation of photosynthesis and respiration may be required to improve model accuracy under stresses (Lizaso *et al.*, 2005).

Photosynthesis and respiration, the main processes determining crop growth rate, are very sensitive to changes in environmental factors. For example, photosynthesis in maize is highly affected by temperatures below 10 °C during the growing season (Naidu *et al.*, 2003). Other factors such as the N status of the plant can be incorporated into leaf-based photosynthesis models.

In the majority of photosynthetic scaling models, the leaf photosynthetic ‘light response’ is the most empirical part of these models (Kull and Kruijt, 1998). One way to capture and describe the effect of N on canopy photosynthesis is by characterizing the photosynthetic light response curve (chapter 1 Fig. 1) at different N levels. In this study, a photosynthesis model based on Collatz *et al.*, (1992) was used and it includes an asymptote of the curve representing the maximum assimilation rate in $\mu\text{mol m}^{-2} \text{s}^{-1}$ (A_{max}), an initial slope of the response to light as $\mu\text{mol CO}_2$ per μmol quanta (apparent quantum efficiency, ϕ), and dark respiration (R_d) expressed in $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Chapter 1 Fig. 1).

In addition to the effect of N on photosynthesis, changes in the photosynthesis light response due to different positions throughout the canopy and leaf age should be taken into account. Since carbon assimilation of the crop also depends on crop N through leaf area development (Gastal and Lemaire, 2001), models that are based on leaf-based photosynthesis require an accurate estimation of leaf area index and canopy architecture (Lizaso *et al.*, 2005)

The objective of this work was to incorporate into a crop growth model a dynamic relationship between photosynthetic parameters and leaf N concentration also taking into consideration, the effects of developmental stage following results from chapter 1. The model used was MaizeGro, which is based on the previously published WINOVAC (Humphries and Long, 1995) and incorporated changes made by Miguez *et al.*, (2009) and Miguez *et al.*, (2011). A leaf area model based on Lizaso *et al.*, (2003) was included and calibrated with field data.

2. Material and methods

2.1 Description of data for model calibration/testing

Measurements of leaf area index (LAI) and individual leaf area were obtained from the field study described in chapter 1 to calibrate and test the LAI model of Lizaso *et al.*, (2003). Leaf level photosynthesis-light response curves were collected from the same experiment at three different N levels (N0, N90, and N225) and throughout the growing season to calibrate the leaf level photosynthesis model from Collatz *et al.*, (1992). Leaf N concentration was measured from the same section in the leaf where the photosynthesis-light curves were performed. More details are available in materials and methods chapter 1.

Carbon allocation was calibrated based on biomass partitioned into leaf, stems and grain obtained from Boyer *et al.*, (unpublished data). In addition, leaf area index and root biomass were not measured in this experiment.

Hourly weather data (solar radiation, maximum and minimum temperature, relative humidity, precipitation, and wind speed) was obtained from Iowa Environmental Mesonet (<http://mesonet.agron.iastate.edu/>).

2.2 Description of the model

The general approach to the dynamic crop model was based on the WIMOVAC model (Humphries and Long, 1995). This version incorporates changes implemented in Miguez *et al.*, (2009) and Miguez *et al.*, (2011). The general crop model was implemented specifically for maize (MaizeGro) in the BioCro R package (version 0.259-9).

Leaf area development

Leaf area of individual leaves was predicted from the model of Lizaso *et al.*, (2003). The model describes three processes of the life cycle of the leaves articulated in a dynamic thermal time

framework: expansion, longevity and senescence. Four discrete functions of simulated leaf-tip number are used for predicting canopy leaf area (Lizaso *et al.*, 2003) Eqns. (A1)-(A3). The green area of the leaves was calculated as the difference between expansion and senescence. Final LAI was calculated as the sum of all green leaves area multiplied by the plant population.

Leaf level photosynthesis

Leaf CO₂ uptake rate (A) was predicted from the steady-state model of Collatz *et al.* (1992) Eqns (A1)–(A4). Tightly coupled with this C₄ photosynthesis model is a version of the Ball *et al.*, (1987) model of stomatal conductance Eqns (A1)–(A3).

The Amax parameter of the photosynthesis model was assumed to depend on the N leaf concentration. This relationship was empirically modeled following Sinclair and Horie (1989):

$$Amax = Amax_m \left(\frac{2}{1 + \exp(-c(Na - Nb))} - 1 \right) \quad \text{Eqn. [1]}$$

where $Amax_m$ is the asymptote (maximum value) of the dependent variable, c is the parameter describing the steepness of the curve, Nb is the intercept of the X -axis denoting a threshold leaf N value at or below Amax equals zero and Na is the current value of the leaf N concentration

During vegetative stages, leaf N concentration was assumed to depend on the leaf (*Leaf*) and stem (*Stem*) biomass as:

$$LeafN = iLeafN (Stem + Leaf)^{-KLN} \quad \text{Eqn. [2]}$$

where KLN is an empirical parameter.

During the reproductive stage Amax was assumed to decrease linearly with thermal time:

$$LeafN = LeafN.R1 * (ThermalT - ThermalT_{R1}) * (slope * 0.045) \quad \text{Eqn. [3]}$$

where *LeafN.R1* is the leaf N concentration at R1 stage, *ThermalT* is the actual thermal time, *ThermalT_{R1}* is the thermal time for R1 and slope is an empirical parameter describing the dependence of the current Amax on thermal time.

Canopy level photosynthesis

The proportion of a canopy that was sunlit and shaded at any point in time was determined based on Norman, (1980) and Forseth and Norman, (1991). The leaf area of sunlit and shaded leaves and the mean irradiance of these two populations were calculated dynamically (Miguez *et al.*, 2009). Sunlit leaves were assumed to receive direct and diffuse solar radiation while shaded leaves received diffuse and scattered light from other leaves in the canopy. Total canopy photosynthesis was the sum of the photosynthesis at both the sunlit and shaded leaves, calculated by the equation for leaf CO₂ uptake. The canopy was divided into 10 layers and the proportion of sun and shade leaves, and their radiative conditions computed for each, following the above principles (Miguez *et al.*, 2009).

The instantaneous transpiration for each leaf class within the canopy was calculated following the approach of Penman–Monteith (Monteith, 1973) and using the stomatal conductance for each layer and sunlit/shaded leaf class following Collatz *et al.* (1992) Transpiration values of both sunlit and shaded leaves at all layers were then summed to give total canopy.

Growth, partitioning and allocation

Carbon allocation was determined by dry matter partitioning coefficients which depend on phenological stages. Partitioning coefficients are set for four different periods during the growing season, V6, V12, R1, and R6. These phenological stages were controlled by thermal periods defined by the sum of the average temperature from the start of the growing season. In this way the fraction of

the available carbon was allocated to each of the plant structural pools, i.e. leaf, stem, root, and grain at the current stage.

The total carbon available for growth during a given developmental stage was the total of net photosynthesis assimilation and leaf/ storage root remobilization. The new leaf area, stem and root length was simulated based on allocated carbon resources for each tissue and the specific leaf area, specific stem length and specific root length, respectively. New leaf growth was assumed to occur uniformly with respect to height in the canopy. Additionally, new stem growth was associated with an increase in canopy height and new root growth with an increase in root density at a specific soil depth (Miguez *et al.*, 2009)

Respiration

Leaf-level respiration is accounted for the leaf-level photosynthesis of Collatz *et al.* (1992). The respiratory cost of maintaining plant structures varied depending on the tissue type (Spain and Keen, 1992). To account for the temperature effect on respiration a Q_{10} of 2 was used. For leaf and stem it was assumed that the proportion was 0.02 and for root it was 0.03.

Soil-plant water relations

The crop model can simulate soil water relations using a layered soil model with hydraulic redistribution but for simplicity a single layer ‘tipping-bucket’ model was used. The single layer soil water model requires parameters of effective rooting depth, field capacity, and wilting point. The model calculates runoff and drainage as well as the fluctuations in available water in the soil. The relationship between available water and water stress was determined by an exponential relationship following (Foley *et al.*, 1996) with minor modifications. The equations are as follow:

$$slp = \frac{1 - WP}{FC - WP} \quad \text{Eqn. [3]}$$

$$intept = 1 - FC * slp \quad \text{Eqn. [4]}$$

$$\Theta = slp * AW + intept \quad \text{Eqn. [5]}$$

$$ws = \frac{1 - \exp^{-2.5 \frac{(\Theta - WP)}{(1 - WP)}}}{1 - \exp^{-2.5}} \quad \text{Eqn. [6]}$$

where FC is field capacity, WP is wilting point, AW is available water. These equations scale theta to be between 0 and 1. The water stress index (ws) also results in a value between 0 and 1.

2.2 Calibration of MaizeGro

Leaf area

Calibration of the maximum leaf area of individual leaves was determined empirically from field measurements described in chapter 1. The equation to calculate the final area of each leaf was fitted to the data to estimate the largest leaf blade (A_{ex}) for each level of N (Eqn. A5) (Lizaso *et al.*, 2003). Leaf longevity (LL) was calculated setting LLx parameter to 800 (°C d, base temperature of 10°C) for N225 and N90 and leaf longevity was adjusted for N0 from Wolfe *et al.*, (1988) (Eqns. A6-A8). Final leaf number (LT) was set equal to 19. Senescence of leaf biomass was simulated by removing portions of the leaf biomass following the approach in Miguez *et al.* (2009) where biomass production is collected and subsequently removed triggered by accumulation of thermal time.

Leaf photosynthesis

The accuracy of photosynthesis estimation by the model (with default values, Collatz *et al.*, 1992) at different growth temperatures was validated using data published by Kim *et al.*, (2007), Crafts-brandner and Salvucci, (2002) and Naidu *et al.*, (2003). The Collatz *et al.*, (1992) model provided a satisfactory fit when this model was used to predict the temperature response obtained in

the three publications by Kim *et al.*, (2007), Crafts-brandner and Salvucci, (2002), and Naidu *et al.*, (2003). We confirmed that the actual functions used for the simulation of temperature are appropriate for currently grown maize hybrids (Appendix C1)

The C₄ photosynthesis model from Collatz *et al.*, (1992) was parameterized for three different N levels throughout the growing season using field data from the experiment described in chapter 1 (Fig. 6). The defaults values from Collatz *et al.*, (1992) were updated and changed according to the leaf N concentration and the developmental stage.

From chapter 1, quantum efficiency (ϕ) was not related with leaf N concentration or developmental stages, for that reason a constant value of $0.0635 \mu\text{mol mol}^{-1}$ was set in the model. Dark respiration (Rd) had a significant but not strong relationship with leaf N concentration thus it was set to $2.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the model. For the maximum assimilation rate (Amax) a non linear relationship with leaf N concentration was included in the model.

Carbon partitioning

Initial values for coefficients for dry biomass partitioning (leaf, stem, and grain) were determined empirically from the measurements of Boyer *et al.*, (unpublished data) collected for two hybrids and two years in Iowa. This parameterization showed good agreement between model simulations and observed dry biomass partitioned into leaf, stem and grain (data not shown). The average root mean square error (RMSE) for leaf, stem, and grain simulations showed that dry biomass production was closely simulated for both hybrids and years (Table 2).

The coefficients obtained from Boyer *et al.*, (unpublished data) were used to test the observed data from the experiment described in chapter 1. Daily solar radiation, temperature, relative humidity, and precipitation were used to simulate dry biomass production.

Table 1 Phenological stages and dry matter biomass partitioning coefficients. Coefficients were calculated based on biomass data from Boyer *et al.*, (unpublished data). Thermal period (TP) is the interval for each phenological stage in thermal time units; the first number is the start of the period and the second is the end.

Stage	TP (°C d)	Leaf	Stem	Root	Grain
V6	0-263	0.59	0.29	0.12	0
V12	264-582	0.59	0.29	0.12	0
R1	583-812	0.27	0.67	0.05	0
R6	813-1656	0	0.01	0.001	0.98

Table 2 Observed and simulated yield (Mg ha⁻¹) for two different hybrids grown in 2007 and 2008, and the average root mean squared error (RMSE) for the dry biomass partitioning components (stem, leaf and grain).

Hybrid	Year	Obs	Sim	RMSE
-----Mg ha ⁻¹ -----				
D	2007	11.8	10.7	1.81
D	2008	9.33	10.1	1.97
A	2007	12.36	11.7	2.1
A	2008	9.89	10.1	1.91

Simulating field data

Simulations conducted for field data corresponding to N0, N90 and N225 were performed using calibrated parameters for LAI, leaf N concentration and partitioning coefficients from previous calibration. The simulation was conducted from planting date to harvest date (130 and 300 day of the year) with a plant density of 8.7 plants m⁻². Thermal time was accumulated with a base temperature of 10 °C, stages R1 and R6 occurred at 750 and 1715 °C d, respectively.

The maximum assimilation rate (A_{max}) was changed according to N concentration following Eqns. [1]-[2], the initial maximum values of A_{max} (A_{max,m}) and the parameter v_{max.b1} were adjusted and optimized for each N level (Table 3). Quantum efficiency was 0.0635 μmol mol⁻¹ and Rd was 2.5 μmol m⁻² s⁻¹. The senescence process in the plant started at 800 °C for green tissue of stem, leaf and root.

The available water was constrained by a field capacity of 29.9%, wilting point of 16.7%, and rooting depth of 1.5 m. One single layer was used and water stress was applied using an exponential function affecting stomata conductance.

Implementation

The main algorithms in WIMOVAC were implemented in the C programming language (Kernighan and Ritchie, 1988), and the interface was written in R (R Development Core Team, 2006). MaizeGro has incorporated the biochemical, physiological, and environmental biophysics mechanism implemented in WIMOVAC, plus parameter estimation capabilities and graphical procedures used to evaluate the agreement between the observed and simulated data.

Table 3 Main parameters included to simulate dry biomass components (leaf, stem, and grain) in maize grown at three different levels of N (N0, N90 and N225).

N Level	Aex	a1	a2	iLeafN	kLN	Amax_m	c	LLx
(Kg ha ⁻¹)	(cm ⁻²)	-	-	g kg ⁻¹	-	(μmol m ⁻² s ⁻¹)	-	(°C d)
N0	553	-4.5	-0.84	35.40	0.240	56	0.569	560
N90	632	-4.8	-0.48	40.84	0.141	56	0.569	750
N225	648	-4.51	-0.089	39.20	0.082	56	0.569	800

3. Results

The observed final area of each leaf was fitted by Eqn. A1 to estimate the area of the largest leaf (A_{ex}) (Fig.1). The area of the largest leaf blade (leaf 13) was 648, 632 and, 553 cm² for N225, N90 and, N0 respectively (Fig 1). The parameters a1 and a2 were also adjusted to improve the fit (Eqn. A5, Table 3).

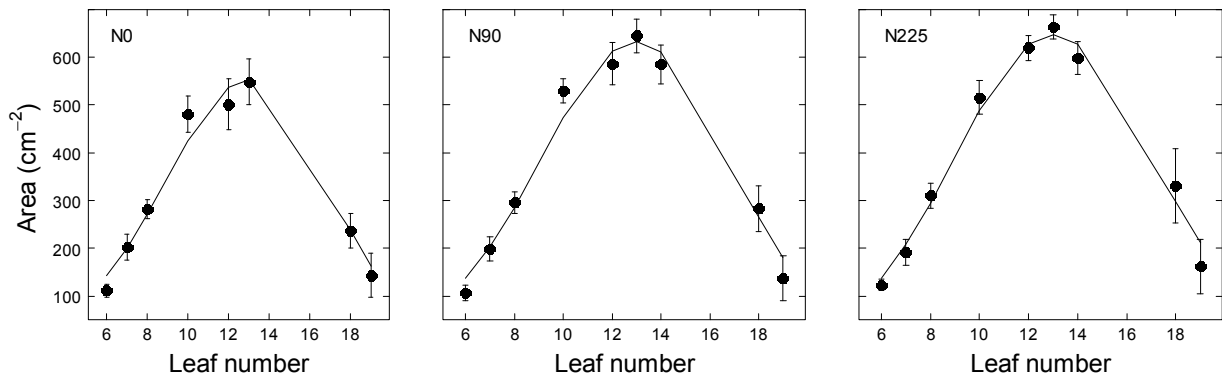


Fig. 1 Observed and simulated maximum area of each leaf (cm² per leaf) for maize grown under three different N treatments (N0, N90 and, N225). The close circles are the observed data from field experiments (chapter 1) and the solid line is simulated using Lizaso *et al.*, (2003) in MaizeGro.

Leaf area model (Lizaso *et al.*, 2003) was fitted to the observed data. Fitted values for N0, N90, and N225 treatments were in general lower than the observed values estimated by the indirect line quantum sensor method (Fig. 2). Differences between the individual leaf model values and the estimated from the line quantum sensor were greater for N90 than N0 and N225 (RMSE, Fig. 2).

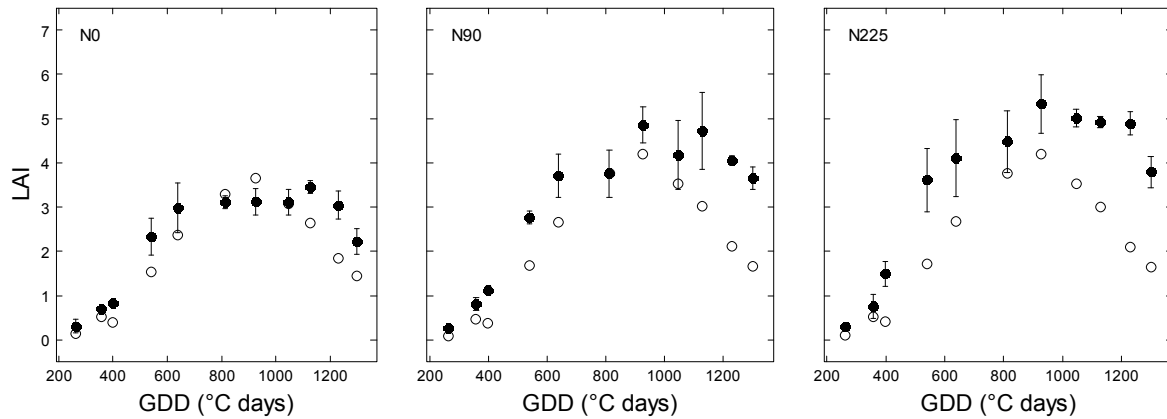


Fig. 2 Observed (close symbols) and simulated (open symbols) leaf area index (LAI) for maize grown at three different N treatments (N0, N90 and, N225). Vertical bars represent the standard error of the mean (n=6). Root mean squared error (RMSE) was 0.16, 0.28, and 0.41 for N0, N90, and N225, respectively.

Leaf N concentration decreased throughout the growing season for the three N treatments measured in the experiment described in chapter 1. Simulated leaf N concentration tended to underestimate leaf N concentration at the beginning of the growing season in particular for N0 and N90 (Fig. 3). During reproductive stages, observed and simulated data closely agreed for N0 and N225 treatment. However, for N90 treatment observations after 800 °C d tended to have an elevation in leaf N concentration that the model did not capture. At the end of the growing season the model tended to overestimate leaf N concentration, observations for N0 and N90 were lower than simulated values.

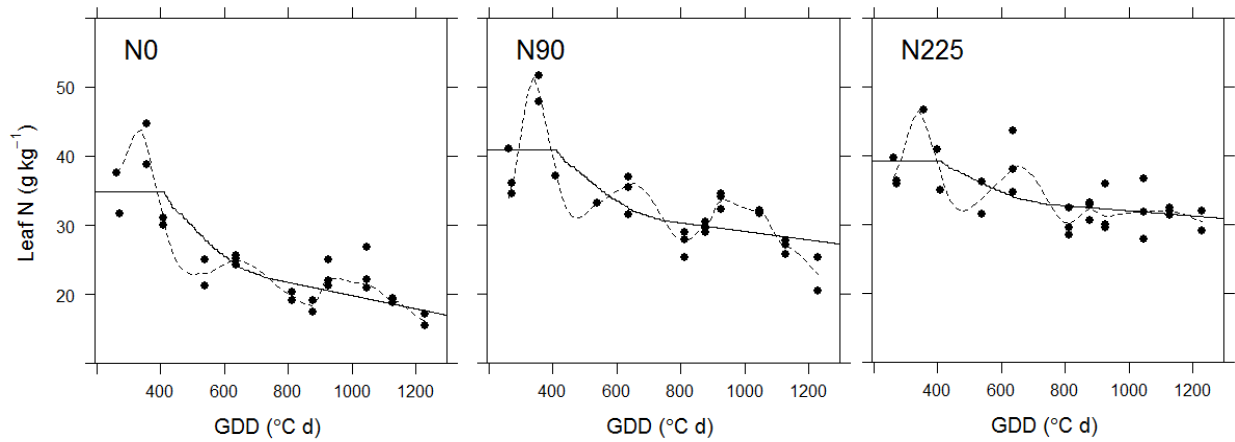


Fig. 3 Observed (close symbols) and simulated (continuous line) leaf N concentration for maize grown at three different N treatments (N0, N90 and, N225) during the growing season (GDD °C d). Dashed line represents a local polynomial fitted to the observed data.

Changes in Amax due to a decrease in leaf N concentration and developmental stage were closely estimated by the model during vegetative stages (Fig. 4). Estimated Amax values for N0 and N90 decreased during vegetative stages whereas for N225 values of Amax remained almost constant. During reproductive stages (after 800 °C d), in particular at the end of the growing season, the model overestimated Amax for N90 and N225. The model, however, was more successful at capturing differences in Amax between vegetative and reproductive stages.

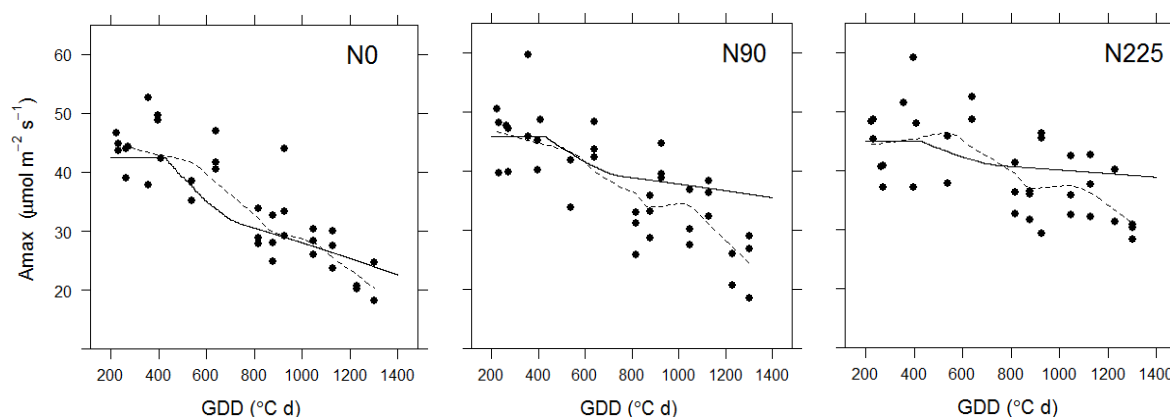


Fig. 4 Observed (close symbols) and simulated (continuous line) Amax for maize grown at three different N treatments (N0, N90 and, N225) during the growing season (GDD °C d). Dashed line represents a local polynomial fitted to the observed data.

Using the parameters presented in Table 3, MaizeGro simulated an increase in total canopy photosynthesis as the leaf area increased for all N treatments reaching the maximum assimilation when leaf area production is completed (around 800 °C d, data not shown). Maximum values of canopy assimilation were 0.060, 0.071, and 0.078 Mg ha⁻¹ hr⁻¹ for N0, N90, and N225, respectively. Differences in canopy assimilation between N225 and N0 were found late in the season when carbon is mainly being partitioned to the grain (after 900 °C d, approximately, Fig. 5).

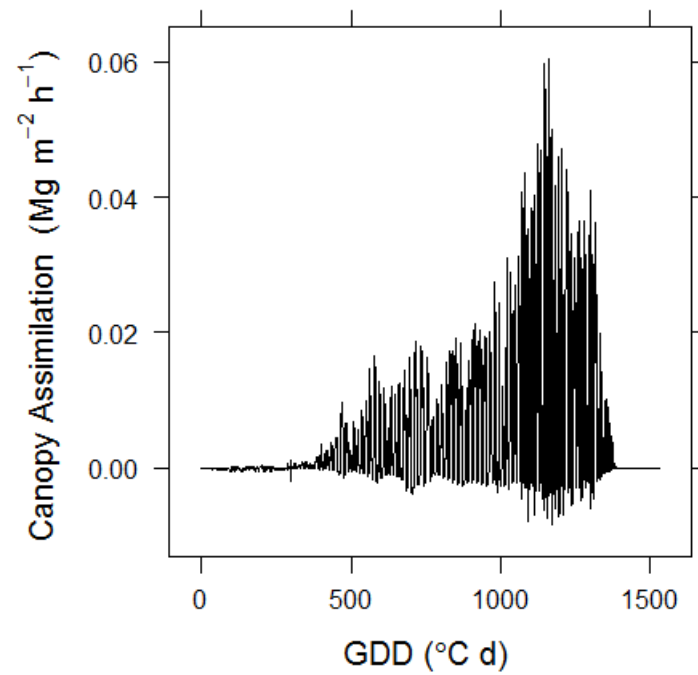


Fig. 5 Differences in canopy assimilation between N225 and N0 simulated by the model during the growing season (GDD °C d).

The parameterization for dry biomass partitioning based on data from Boyer *et al.*, (unpublished data) showed an acceptable agreement between model simulations and final grain yield values from the field experiment described in chapter 1 (Table 4). In terms of leaf and stem biomass accumulation, maximum values of biomass simulated by the model were lower than the dry biomass obtained at maturity from the field experiment described in chapter 1. The dynamics of dry biomass components are shown in Appendix C2 (for N225).

Table 4 Observed and simulated dry biomass for leaf, stem and grain (Mg ha^{-1}) for three different N levels (N0, N90, and N225) at the field experiment described in chapter 1.

N Level	Leaf		Stem		Grain	
	Obs.	Sim.	Obs.	Sim.	Obs.	Sim.
kg ha^{-1}	Mg ha^{-1}					
N0	2.9	1.9	5.3	3.7	7.2	7.46
N90	3.4	2.2	6.1	4.4	12.3	12.0
N225	3.6	2.3	6.7	5.0	13.8	13.3

4. Discussion

Leaf area influences interception and utilization of solar radiation of maize crop canopies and consequently maize dry matter accumulation and grain yield. MaizeGro computes dry matter accumulation from temporal integration of canopy photosynthesis. Therefore leaf area and number are important factors in the estimation of canopy photosynthesis (Boote *et al.*, 1996).

The indirect measurements of LAI using the line quantum sensor and the simulated LAI by the model (Lizaso *et al.*, 2003) did not fully agree (Fig. 2). Leaf area index estimated by the model was lower than the observed mainly for N90 and N225 treatments. According to Lizaso *et al.*, (2003), leaf area estimation is very sensitive to three main parameters, LT (final number of leaves), Aex (area of the largest leaf) and LLx (longevity of the most longevous leaf). Lizaso *et al.*, (2003) showed through a sensitivity analysis that LT in particular, became very important variable for accurate prediction of leaf area. A difference in one leaf had a significant impact on the maximum leaf area predicted. In this study, however, the final leaf number was set up according to the real number observed in the field (LT=19).

In addition, Aex (area of the largest leaf) and LLx (longevity of the most longevous leaf) also influenced simulation of leaf extension and senescence (Lizaso *et al.*, 2003). The parameter Aex was successfully estimated (Eqn. A1, Fig. 1), even though the maximum LAI predicted by the model was lower than the observed values (Fig. 2, $GDD \geq 800$ °C d).

On the other hand, LAI measured by the line quantum sensor seemed to increase after 800 °C d (R1 stage) which is unlikely. After R1 all leaves are fully expanded and plants are at maximum height (Abendroth *et al.*, 2011). Consequently, the disagreement between the indirect measurement of LAI and what it should be obtained by a destructive method may be, in part, caused by a methodological issue for the LAI measurements during reproductive stages. The maximum measurable LAI is generally lower for these devices measuring gap fraction than the one assessed via direct methods, with LAI reaching an asymptotic saturation level at a value of about 5. The likely

cause is the gap fraction saturation as LAI approaches 5–6 (Gower *et al.*, 1999). Interestingly, we would expect the values obtained after R1 to be lower (not higher) as it has been found in the literature (Wilhelm *et al.*, 2000).

A methodological issue with LAI estimation by the line quantum sensor can be considered also early in the season. Before canopy closure, placing the line quantum sensor between rows can overestimate LAI (Fig. 2). For a more representative estimation of LAI, one single measurement should consist in several sub measurements placing the bar at 0, 0.25, 0.50, and 0.75 between rows (Wilhelm *et al.*, 2000). Furthermore, the line quantum sensor does not discriminate between leaf, stem, ear tissue or dead LAI; all plant parts are counted as leaf area in proportion to the amount of light they intercept. In contrast, the model based on Lizaso *et al.*, (2003) estimates the total amount of green area without considering other plant structures. The differences in definition of leaf area between the methods suggest that the LAI meter would overestimate LAI.

The calibrated model not only included LAI production at different N treatments, but it also incorporated a relationship between Amax and leaf N concentration at vegetative and reproductive stages. The relationship between leaf N concentration and thermal time for three different N levels (N0, N90, and N225) was calibrated based on field data described in chapter 1 (Chapter 1 Fig. 5, Eqn. 1). The function implemented in the model closely described the decrease in N concentration during vegetative stages. In contrast, during reproductive stages the model tended to overestimated leaf N concentration for N0 and N90 (Eqn. A11, Fig. 3).

There are several ways in which the N content of leaves can be varied in order to investigate relationships with photosynthesis rates; in general they all give similar relationships (Evans, 1983; Field and Mooney, 1986). Leaf N content can vary according to canopy position, leaf age, the photosynthetic photon flux density (PPFD) under which the plant is grown and soil N supply.

Measurements performed in this work were obtained from different leaves during vegetative stages and on the leaf 14 (ear leaf) during reproductive stages. Although, leaf N concentration decreased during the growing season there were some fluctuations in leaf N concentration that is likely a result of the balance of N demand and supply at the individual leaf level. Also, leaf N concentration during vegetative stages was measured on expanded leaves, but the accumulation of N might still increase after this point in addition to being influenced by translocation of N from senescing leaves (Lambers *et al.*, 2008).

During reproductive stages leaf N concentration tended to increase after 800 °C d in particular for N0 and N90. The demand for N during reproductive stages is especially high for the ear leaf (Muchow, 1994) and, under non limiting N conditions (N225) leaf N concentration was maintained almost constant and no translocation from other leaves may be occurring. For N0 and N90, however, the increase in leaf N concentration after 800 °C d could be explained by a translocation of N from senescing leaves and stem to the ear leaf due to high demand for N from the grains (Ta and Weiland, 1992)

A way to improve the characterization of leaf N concentration could be to couple the dynamics of leaf N concentration on individual leaves with individual leaf area production following the model by Lizaso *et al.*, (2003). Furthermore, the dynamic of leaf N content expressed on a mass basis could be replaced by area basis (specific leaf nitrogen, SLN). Several authors have reported that for data that include N deficient plants, the correlation between photosynthesis rates and N content may be greater when both are expressed on a leaf dry matter basis simply because of variation in specific leaf weight (SLW) by growth environment, development, nutrition, etc. (Gulmon and Chu, 1981; Hirose and Kitajima, 1986). When leaf N content varies as a result of PPFD during growth (including canopy position and seasonal effects), leaf area-based measurements tend to give better correlations than those based on leaf dry matter (DeJong and Doyle 1985; Reich *et al.*, 1991).

The biomass allocated to the grain closely agreed with observed yields for all N treatments (Table 4). There were discrepancies, however, between observed and simulated maximum dry biomass for leaf and stem. Comparison between observed and predicted leaf and stem dry biomass allocation indicated that simulations were 35% lower than observed values for maximum dry biomass in leaf and stem. It is difficult to attribute this discrepancy to any one factor. Biomass allocation was based on Boyer *et al.*, (unpublished data) and no systematic bias was observed in this case. The water stress factor implemented in the model, however, may require further calibration and validation to adjust the reduction in carbon assimilation by the canopy.

5. Conclusions

The objective of the present work was to link the current parameterization of the photosynthesis model (Collatz *et al.*, 1992) with a dynamic of leaf N concentration. In addition, a leaf area model (Lizaso *et al.*, 2003) was calibrated for three levels of N (N0, N90, and N225). Leaf area production was successfully estimated by the model considering that the model was calibrated with LAI recorded by an indirect method.

The effect on leaf N concentration in Amax was better simulated during vegetative stages than reproductive stages. The model, however, captured differences in Amax between vegetative and reproductive stages. Further improvements can be performed to the dynamics of N for individual leaves.

By improving the ability of dynamic models to capture the effect of N on biochemistry and biophysics process, the extent of applicability of these models can be increased especially to test specific crop characteristics in plant breeding programs and N management practices.

GENERAL CONCLUSIONS

A characterization and parameterization of leaf area index and photosynthetic parameters for three different N levels has been presented in this work. Leaf area index and light interception of solar radiation were reduced by a decrease in individual leaf area, when no N or 90 kg ha⁻¹ were applied. The Amax parameter was related to leaf N concentration during vegetative and reproductive stages. Dark respiration (Rd) showed a significant, although not strong, relationship with leaf N concentration. Apparent quantum efficiency (ϕ) was found to be 0.0635 $\mu\text{mol mol}^{-1}$ on average and was not affected by leaf N concentration or developmental stage. The variability observed in field experiments, however, suggested that more research is needed to characterize changes in ϕ to estimate photosynthesis under low irradiance, a better estimation of the light absorption by the leaf can be an important factor to understand the variability in ϕ .

The leaf area index model (Lizaso *et al.*, 2003) calibrated for each level of N and the parameterization of the photosynthesis model (Collatz *et al.*, 1992) linked with the dynamic of leaf N concentration were included in MaizeGro. The calibration of the dry biomass partitioning coefficients was based on modern maize hybrids, and an accurate estimation of the root biomass partitioning is still needed. In addition, the leaf area model can be improved further by a better estimation of field LAI values and measurements of leaf longevity of individual leaves.

Dynamic crop growth models capable of capturing the effect of N on biochemistry and biophysics process would improve the assessment of N supply and demand in the cropping systems that might alleviate the impact of excessive N use on the environment.

Appendix A The key equations in MaizGro

$$M = \min \frac{(A_{\max} + \phi I_{\text{abs}}) \pm \sqrt{(A_{\max} + \phi I_{\text{abs}})^2 - 4(A_{\max} + \phi I_{\text{abs}})\theta_{\text{curve}}}}{2\theta_{\text{curve}}} \quad (\text{A1})$$

$$A_{\text{gross}} = \min \frac{(M + k_t(\frac{C_i}{P})) \pm \sqrt{(M + k_t(\frac{C_i}{P}))^2 - (4Mk_t\beta)}}{2\beta} \quad (\text{A2})$$

$$A_n = A_{\text{gross}} - R_d \quad (\text{A3})$$

$$V_t = \frac{A \frac{T_l - 25}{\max Q_{10} \frac{10}{10}}}{(1 + e^{0.3(13 - T_l)})(1 + e^{0.3(T_l - 36)})} \quad (\text{A4})$$

$$A_{ei} = A_{ex} * e^{a1(\frac{(LN_i - LN_x)}{(LN_x - 1)})^2 + a2(\frac{(LN_i - LN_x)}{(LN_x - 1)})^3} \quad (\text{A5})$$

$$LL_i = L0 + LLx * e^{\frac{-(LN_i - LN_L)^2}{2W_l^2}} \quad (\text{A6})$$

$$LN_L = 3.59 + 0.498LT \quad (\text{A7})$$

$$w_l = \frac{1}{3}LT \quad (\text{A8})$$

$$GR_{si} = (A_{si} * K_{si})e^{-k_{si} \frac{t - t_{si}}{(1 + e^{-K_{si}(t - t_{si})})^2}} \quad (\text{A9})$$

Appendix B Abbreviations

I_{abs} = Photon flux absorbed by either sunlit or shaded leaves within a canopy layer ($\mu\text{mol m}^{-2} \text{s}^{-1}$)

θ_{curve} = Curvature parameter (dimensionless)

A_{gross} = Gross rate of CO_2 uptake per unit leaf area ($\mu\text{mol mol}^{-1}$)

k_t = C_4 slope factor

P = Leaf surface partial pressure of CO_2 (kPa)

β = C_4 curvature parameter

A_{ei} = Expanded area of the i th leaf blade (cm^2)

A_{ex} = Expanded area of the largest leaf blade (cm^2)

a_1 = Shape parameter controlling curve

LN_i = Nodal position of the i th leaf blade

LN_x = Nodal position of the largest blade leaf

LL_i = Longevity in the i th leaf blade (GDD)

L_0 = Asymptote

LL_x = Longevity of the most longevous leaf (GDD)

LN_L =Nodal position of the most longevous leaf

W_l = Parameter controlling the curve leaf

LT = Total number of leaves

GR_{si} = Senescence rate of the i th leaf blade ($\text{cm}^2 \text{GGD}^{-1}$)

As_i =Senesced area of the i th leaf blade (cm^2)

t = Thermal time (GDD)

ts_i = Thermal time when the i th leaf blade reaches 50% of As_i (GDD)

Ks_i = Parameter controlling the slope of the senescence of the i th leaf blade

Appendix C Additional figures

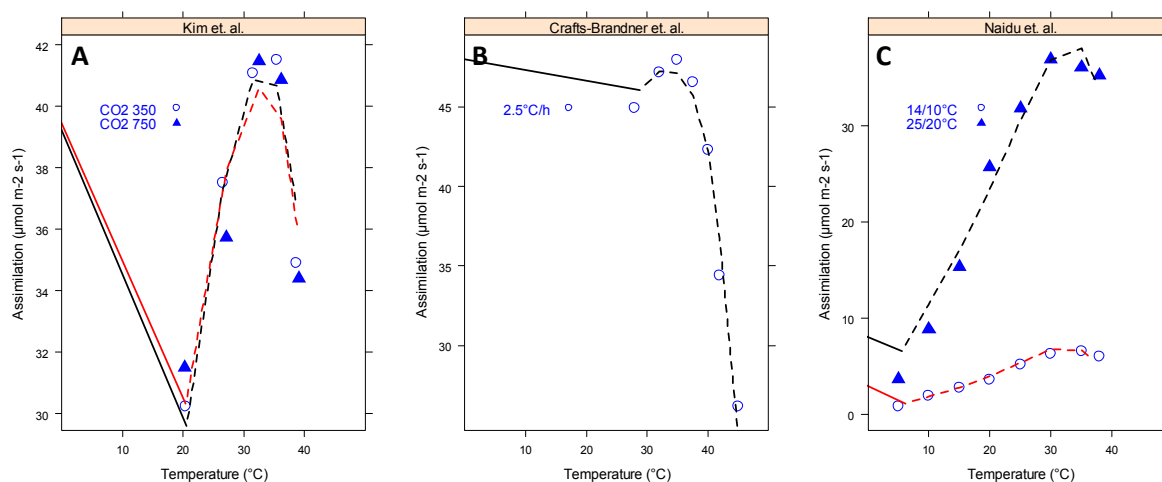


Fig. C1 Response of carbon assimilation ($\mu\text{mol m}^{-2} \text{s}^{-1}$) to temperature ($^{\circ}\text{C}$) for Maize. A) Observed data from Kim *et al.* (2007) for ambient and elevated CO_2 . B) Data from Crafts-Brandner and Salvucci (2002) for $2.5^{\circ}\text{C h}^{-1}$ temperature increase treatment. C) Observed data from Naidu *et al.* (2003) for low and warm growth temperatures treatments. The dotted line is simulated using the Collatz *et al.* (1992) model.

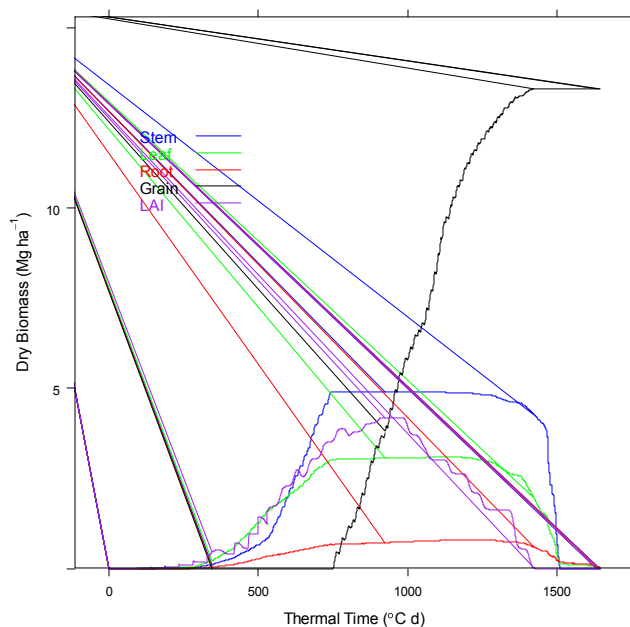


Fig. C2 Simulated dry biomass partitioning and leaf area index (LAI) for maize growing at N200 treatment for data from site11 through the growing season.

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